IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:

Duft, *et al.*

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For: METHODS FOR TREATING OBESITY

Group Art Unit: 1645

Examiner: S. Devi

DECLARATION OF ANDREW A. YOUNG

Commissioner for Patents
Washington, DC 20231

Sir:

I, Andrew A. Young, declare that:

1. I am employed by Amylin Pharmaceuticals, Inc. as its Vice President of Research. I have been employed by Amylin Pharmaceuticals since 1989, and have held my current position since 1998. I was hired as Director of Physiology, promoted to Senior Director of Physiology, then Vice President of Physiology, and finally to my current position.

2. I hold the following degrees: Bachelor of Science (Human Biology), University of Auckland, 1974; Master of Science with Honours (First Class) in Physiology, University of Auckland, 1978; Bachelor of Medicine, Bachelor of Surgery (medical qualification), University of Auckland, 1979; recognized by US Educational Commission for Foreign Medical Graduates (ECFMG), 1983; Doctor of Philosophy in Physiology, University of Auckland, 1985. I have conducted research in the area of physiology and metabolism since 1976, and have specialized in the field of diabetes since 1984. I am an inventor on ten issued patents in the field of diabetes and metabolic disease.

3. A study was performed under my supervision to evaluate the effects of infusion of rat amylin on food intake and body weight in C57BL/6 Mice. It showed that, compared to controls, administration of amylin resulted in reduced body weight in spite of the fact that there was no overall reduction in food intake.

4. In the study, the effects of continuous infusion of rat amylin were determined in C57BL/6J mice (Jackson Labs, Bar Harbor ME). The mice gained body weight by *ad libitum* feeding of a high-fat diet (58 % kcal fat; HF; Research Diets, Inc., New Brunswick, NJ) instead of a standard low-fat (11 % kcal fat; LF; Research Diets, Inc.) beginning at 4 weeks of age. Five weeks after diet introduction, each mouse was implanted subcutaneously with an osmotic pump (Durect, Inc.), which delivered 30, 100 or 300 µg rat amylin / kg body weight / day continuously for 4 weeks. The study included the following five treatment groups (n relates to data sets for food intake measurement and body mass, respectively): (i) ***Low-fat diet + vehicle*** via osmotic pump (n=8, 17); (ii) ***High-fat diet + vehicle*** via osmotic pump (n=8, 16); (iii) ***High-fat diet + amylin 30µg/kg/day*** via osmotic pump (n=7, 14); (iv) ***High-fat diet + amylin 100µg/kg/day*** via osmotic pump (n=7, 14); and, (v) ***High-fat diet + amylin 300µg/kg/day*** via osmotic pump (n=7, 14). Animals were maintained on the experimental diets throughout the rat amylin treatment period. Cumulative food consumption and body weights were recorded weekly.

5. The results of the study showed the effects of a high fat diet and the effects of amylin treatment in high-fat fed mice. Regarding the effects of a high-fat diet, mice fed a high-fat diet and treated with saline did not consume significantly more energy over 4 weeks than mice fed a low-fat diet (644±8 vs 639±29 kcal). By 4 weeks of treatment, however, they had gained more body weight (3.29±0.19g vs 2.60±0.15g, respectively). Regarding the effects of amylin treatment in high-fat fed mice, total kcal of diet consumed over the 4 weeks of treatment did not differ

significantly between treatment groups (644±8, 637±12, 643±11, 631±22 kcal for groups ii to v, respectively).

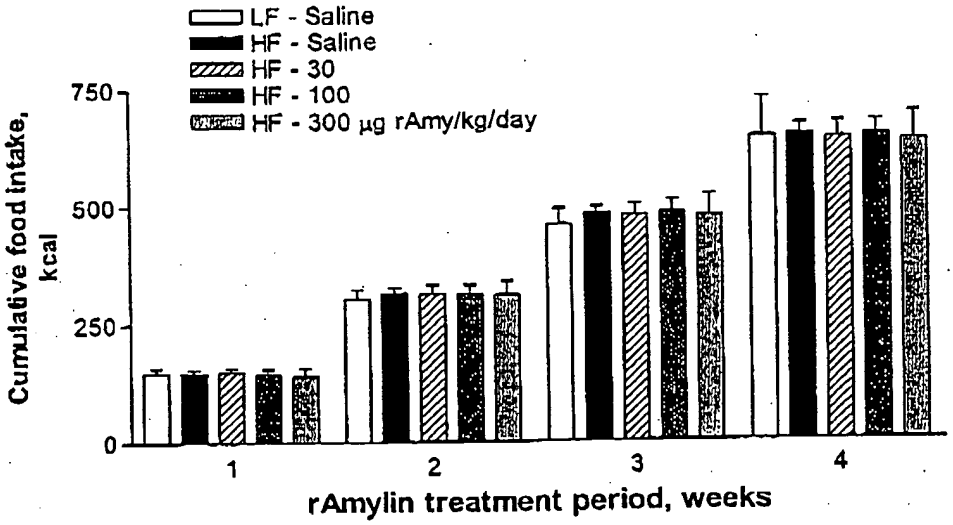
6. The study results indicate that amylin administration did not affect total energy consumed over 4 weeks. However, infusion of 100 μg or 300 μg rat amylin/kg body weight/day caused a significant and dose-dependent decrease in body weight gain in fattened C57BL/6 mice (see Figures 1 and 2). Decreased weight gain was detectable by 2 weeks of treatment and was sustained throughout the 4-week treatment period. In other words, treatment of experimental animals with rat amylin, an amylin agonist, did not affect long term food intake, while it did dose-dependently and potently decrease weight gain in fattened, high-fat fed C57Bl/6 mice. Thus, this model shows that the effects of amylin to ameliorate weight gain are not predicted by, or obligatorily linked to, an effect on food intake.

7. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Dated: October 22, 2001

Andrew A. Young, M.D., Ph.D.

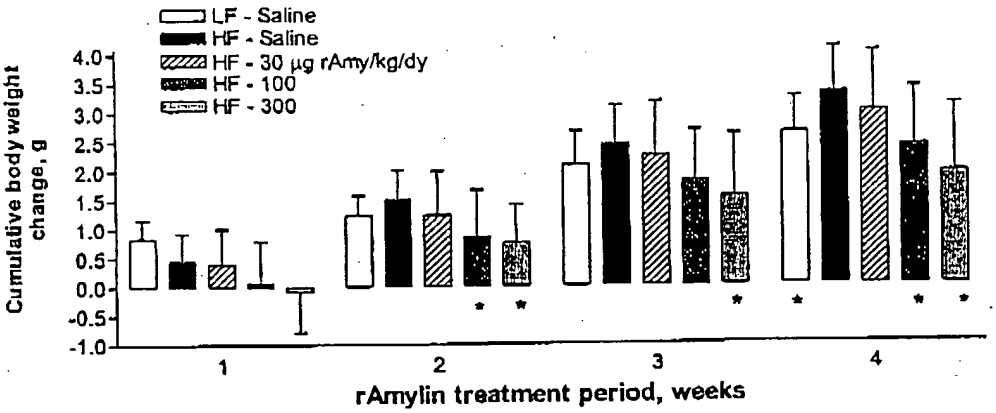
Food intake during 4 weeks of rAmylin infusion in fattened C57Bl/6 mice



C57Bl/6 mice were fed high fat (HF; 58% of dietary kcal as fat) or low fat (LF; 11% of dietary kcal as fat) chow. After 5 weeks on chow, each mouse was implanted with an osmotic pump which delivered the indicated daily dose of rat amylin continuously for 4 weeks. Body weight and food intake were measured biweekly. Each bar represents the mean ± sd of n=7-8 cages; 2 mice/cage.

Figure 1

Body weight change during 4 weeks of rAmylin infusion in fattened C57Bl/6 mice



C57Bl/6 mice were fed high fat (HF; 58% of dietary kcal as fat) or low fat (LF; 11% of dietary kcal as fat) chow. After 5 weeks on chow, each mouse was implanted with an osmotic pump which delivered the indicated daily dose of rat amylin continuously for 4 weeks. Body weight and food intake were measured biweekly. Each bar represents the mean ± sd of n = 14 - 16 mice.

* indicates signficant difference from HF - saline control group; ANOVA, $p<0.05$, Dunnett's test.

Figure 2